

We claim:

1. A method for the preparation of an alcoholic extract from *Rhizoma Chuanxiong* useful as a progestogen, said method comprising subjecting powdered *Rhizoma Chuanxiong* to a first extraction with an alcoholic solvent selected from the group consisting of ethanol and methanol, separating a first *Rhizoma Chuanxiong* extract obtained thereby from a supernatant, subjecting the filtered first extract to a second extraction with an alcoholic solvent to obtain a second extract, separating and drying the second extract.
2. A method as claimed in claim 1 wherein the solvent used for the first extraction is selected from ethanol in a concentration of 70% and 100%.
3. A method as claimed in claim 1 wherein the solvent for the first extraction is selected from methanol in a concentration of 70% and 100%.
4. A method as claimed in claim 1 wherein in the first extraction, the powdered *Rhizoma Chuanxiong* is mixed with the solvent and then allowed to soak for 5-7 days at 37°C.
5. A method as claimed in claim 1 wherein the first extract is separated from the supernatant by filtration using Whatman Grade I (11µm pore size) filter paper.
6. A method as claimed in claim 1 wherein the second extraction is carried out over a time period in the range of 2-3 hours.
7. A method as claimed in claim 1 wherein the second extract is separated from the solvent by filtration.
8. A method as claimed in claim 1 wherein the separated second extract is dried in a rotary evaporator.
9. A method as claimed in claim 1 wherein the solvent used for the second extraction is the same as the solvent used for the first extraction.
10. A method as claimed in claim 1 wherein the solvent used for second extraction is selected from the group consisting of 50%, 70% and 100% ethanol in water; and 70% and 100% methanol in water.
11. A method as claimed in claim 1 wherein the dried second extract is suspended in an alcoholic medium.
12. A method as claimed in claim 11 wherein the medium used for suspending the dried second extract is selected from ethanol and methanol in a concentration of 100%.
13. A method as claimed in claim 11 wherein the dried extract is suspended in the alcoholic medium at a concentration in the range of 6.25µg/ml to 100 µg/ml of medium.
14. A method as claimed in claim 13 wherein the dried extract is suspended in the alcoholic medium at a concentration of 50 µg/ml.

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15. A method as claimed in claim 1 wherein the dried extract of *Rhizoma Chuanxiong* contains 3-butyl-4,5-dihydrophthalide and 3-butyl-phthalide as active constituents.
16. A method as claimed in claim 15 wherein the 3-butyl-4,5-dihydrophthalide is present in the extract in an amount of about 95% and the 3-butyl-phthalide is present in an amount of about 2%.
17. A method as claimed in claim 1 wherein the dried second extract of *Rhizoma Chuanxiong* is subjected to purification to enrich the second extract.
18. A method as claimed in claim 17 wherein the purification is carried out by eluting the dried second extract with a solvent using reverse phase extraction in a C18 matrix contained in a glass column.
19. A method as claimed in claim 18 wherein the solvent used for elution is selected from the group consisting of 30% methanol, 80% methanol, 100% methanol and DCM.
20. A method as claimed in claim 17 wherein the purification is carried out using reverse phase extraction in a Diol matrix contained in a glass column.
21. A method as claimed in claim 20 wherein the reverse phase extraction is carried out using an eluate selected from the group consisting of 30%EtoAc:70%DCM, 100%DCM and 60%EtoAc:40%DCM.
22. A method as claimed in claim 17 wherein the dried extract is first subjected to solvent/solvent partitioning to remove tannins present in said extract.
23. A method as claimed in claim 22 wherein the solvent/solvent partitioning is carried out by first mixing the dried second extract in ethanol, mixing the ethanolic extract obtained thereby with water to obtain an ethanolic solution, adding hexane to the solution to obtain a first hexane layer, separating the hexane layer and adjusting the ethanol:water ratio in the remaining solution to 3:2, adding equivolume of DCM to obtain a DCM layer, separating the DCM layer, adding butanol to the remaining solution after DCM layer removal to obtain a butanol layer, separating the butanol layer to leave a water layer as remnant, the tannins being partitioned to the butanol layer and the water layer, the prior separated hexane and DCM layers being enriched in *Rhizoma Chuanxiong* extract.
24. A method as claimed in claim 17 wherein the purification comprises subjecting the crude extract to HPLC.
25. Therapeutic method for progesterone replacement or supplementation comprising administering to a patient suffering from conditions requiring progesterone replacement or supplementation, a therapeutically effective dose of an extract of *Rhizoma Chuanxiong*.

26. A method as claimed in claim 25 wherein the therapeutically effective dose of *Rhizoma Chuanxiong* extract is in the range of 6.25µg/ml to 100µg/ml.
27. A method as claimed in claim 25 wherein the therapeutically effective dose of *Rhizoma Chuanxiong* extract is 50µg/ml of crude extract.
- 5 28. A method as claimed in claim 25 wherein the conditions requiring replacement/supplementation of progesterone are selected from the group consisting of menstrual disorders, amenorrhoea, menorrhagia, polycystic ovarian syndrome, pregnancy complications, endometriosis, contraception, menopause, endometrial hyperplasia and hormonal replacement.
- 10 29. A method as claimed in claim 25 wherein the *Rhizoma Chuanxiong* extract is used alone or in combination with co-elutes from *Rhizoma Chuanxiong*.
30. A method as claimed in claim 25 wherein the *Rhizoma Chuanxiong* extract is administered orally.
- 15 31. A method as claimed in claim 25 wherein the *Rhizoma Chuanxiong* extract is administered subcutaneously.
32. A method for the treatment of stroke or brain injuries in a subject suffering the same comprising administering to the subject a pharmaceutically effective amount of *Rhizoma Chuanxiong* extract. A method as claimed in claim 32 wherein the pharmaceutically effective dose of *Rhizoma Chuanxiong* extract is in the range of 6.25µg/ml to 100µg/ml.
- 20 33. A method as claimed in claim 32 wherein the pharmaceutically effective dose of *Rhizoma Chuanxiong* extract is 50µg/ml of crude extract.
34. A method as claimed in claim 32 wherein the *Rhizoma Chuanxiong* extract is used alone or in combination with co-elutes from *Rhizoma Chuanxiong*.
- 25 35. A method as claimed in claim 32 wherein the *Rhizoma Chuanxiong* extract is administered orally.
36. A method as claimed in claim 32 wherein the *Rhizoma Chuanxiong* extract is administered subcutaneously.
37. A kit for use in conducting progesterone receptor assay comprising HeLa cells transiently co-transfected with a first plasmid and a second using lipofectamine.
- 30 38. A kit as claimed in claim 38 wherein the first plasmid comprises of DNA encoding the full length human progesterone receptor (PR-B) and the second plasmid comprises a progesterone reporter gene (PRE2-TATA-LUC) comprising a luciferase reporter gene driven by two copies of the progesterone response element from the aminotransferase gene.

39. A method for the preparation of a progesterone receptor assay comprising growing HeLa cells in 24-well microtiter plates, infecting the grown He-La cells with a first plasmid and a second plasmid using lipofectamine, the first plasmid comprising of DNA encoding the full length human progesterone receptor (PR-B), and the second plasmid comprising a progesterone reporter gene (PRE2-TATA-LUC) comprising a luciferase reporter gene driven by two copies of the progesterone response element from the aminotransferase gene, exposing the transfected cells to ligands in RPMI 1640 medium, supplemented with 10% charcoal-stripped fetal calf serum, 2mM L-glutamine, 0.1mM non-essential amino acids and 1mM sodium pyruvate for 42-48 hours at 37°C in a 5% carbon dioxide incubator, exposing the replicate wells to ethanol vehicle, rinsing the cells with a buffer selected from a PBS buffer and lysis buffer, collecting the cell lysates for measurement of luciferase activity.
40. A method for conducting assay of progestogenic activity of an extract *Lingusticum chuanxiong* comprising exposing an assay comprising transfected cells of He-La transiently co-transfected with a first plasmid and a second using lipofectamine, to increasing doses of progesterone and the extract.
41. Use of a therapeutically effective dose of an extract of *Rhizoma Chuanxiong* for progesterone replacement or supplementation by administering the extract to a patient suffering from conditions requiring progesterone replacement or supplementation.
42. Use as claimed in claim 42 wherein the therapeutically effective dose of *Rhizoma Chuanxiong* extract is in the range of 6.25µg/ml to 100µg/ml.
43. Use as claimed in claim 42 wherein the therapeutically effective dose of *Rhizoma Chuanxiong* extract is 50µg/ml of crude extract.
44. Use as claimed in claim 42 wherein the conditions requiring replacement/supplementation of progesterone are selected from the group consisting of menstrual disorders, amenorrhoea, menorrhagia, polycystic ovarian syndrome, pregnancy complications, endometriosis, contraception, menopause, endometrial hyperplasia and hormonal replacement.
45. Use as claimed in claim 42 wherein the *Rhizoma Chuanxiong* extract is used alone or in combination with co-elutes from *Rhizoma Chuanxiong*.
46. Use as claimed in claim 42 wherein the *Rhizoma Chuanxiong* extract is administered orally.
47. Use as claimed in claim 42 wherein the *Rhizoma Chuanxiong* extract is administered subcutaneously.

48. Use of a pharmaceutically effective dose of an extract of *Rhizoma Chuanxiong* for the treatment of stroke or brain injuries in a subject suffering the same comprising administering to the subject a pharmaceutically effective amount of *Rhizoma Chuanxiong* extract.
- 5 49. Use as claimed in claim 49 wherein the pharmaceutically effective dose of *Rhizoma Chuanxiong* extract is in the range of 6.25µg/ml to 100µg/ml.
50. Use as claimed in claim 49 wherein the pharmaceutically effective dose of *Rhizoma Chuanxiong* extract is 50µg/ml of crude extract.
- 10 51. Use as claimed in claim 49 wherein the *Rhizoma Chuanxiong* extract is used alone or in combination with co-elutes from *Rhizoma Chuanxiong*.
52. Use as claimed in claim 49 wherein the *Rhizoma Chuanxiong* extract is administered orally.
- 15 53. Use as claimed in claim 49 wherein the *Rhizoma Chuanxiong* extract is administered subcutaneously.